Cellular Immunotherapy for Cancer

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Objectives

- explain the rationale behind cellular adoptive immunotherapy
- describe methods of improving cellular adoptive immunotherapy
- identify mechanisms of tumor escape from cellular adoptive immunotherapy
The Immune System

Innate
- skin, mucosal barriers
- complement
- neutrophils, NK cells, mast cells, basophils, eosinophils

Adaptive
- T cell mediated
  - CD8 cytotoxic
  - CD4 helper
- B cell mediated
  - antibody production
Immunotherapy for Cancer

- **Active**
  - vaccines

- **Passive**
  - cell transfer strategies
    - "Adoptive Immunotherapy"
Cancer as an immune target

Evidence
- unique cancer antigens
- immunosuppression
- “immune editing”¹ –
  - elimination phase
  - equilibrium phase
  - escape phase

¹Immunity 2004 21(2):137-148
Benefits of Immunotherapy

- relatively non-toxic therapy
- exquisitely specific
- curative/adaptive
- long lived protection/memory
Immunotherapy for Cancer

Passive cell transfer strategies
“Adoptive Immunotherapy”

1960’s: early animal models
1970’s: Immuno-suppression required
1980’s: IL-2, LAK cells
1990’s: TIL cells, TDLN cells
2011
Advantages of Passive Cellular Approach

**Pros**
- feasible
- activation and expansion without dampening factors
- supraphysiologic numbers of cells
- ex vivo genetic manipulation of cells

**Cons**
- labor intensive
- expensive
- too artificial(?)
- transient cell viability
- no ‘off-the-shelf’ ability
Advantages of Passive Cellular Approach
Advantages of Passive Cellular Approach
Immunotherapy for Cancer: 1970-1980

- Rosenberg et al\(^2\). – cultured mononuclear fraction of peripheral blood (leucopheresis) with IL-2
- generated lymphokine-activated killer cells (LAK)
- LAKs found to be NK cells
- when given with exogenous IL-2 – clinical responses
- improved with cyclophosphamide or TBI\(^3,4\)

\(^2\)Cancer Res 1981; 41: 4420-4425.
\(^4\)Science 1986; 233: 1318-1321.
- Rosenberg et al. – cultured tumor suspension in IL-2
- generated tumor-infiltrating lymphocytes (TIL)
- TILs determined to be mostly CD8 T cells
- when given with exogenous IL-2 – clinical responses
- 50-100x more effective than LAK cells

Immunotherapy for Cancer: 1990-2000

- optimal source of T cells?
  - tumor draining lymph nodes (TDLN)
  - vaccine primed lymph nodes (VPLN)

2 major areas –
Host – limits of immunodepletion prior to adoptive transfer
Effector – Genetically engineered T cells
Immunodepletion prior to adoptive transfer

- eliminate competition for cytokines\textsuperscript{7}
- eliminate suppressor cells (Tregs)
- generates maturation of dendritic cells
- increase in tumor antigen display

Immunocompetent host

- Immature DC
- Adoptively transferred tumor-specific CD8
- Secreted cytokines (IL-2, IL-7, IL-15)
- Tumor cells
Non-myeloalative preconditioning
Myeloalative preconditioning

Mature DC

Adoptively transferred tumor-specific CD8^+ 

Fully activated tumor-specific CD8^+

tumor cells
Genetically engineered T cells

- co-stimulation is complex and necessary
Antigenicity vs. Immunogenicity

Antigen: APC T cell interaction, no costimulation
Result – T cell anergy, apoptosis, or Suppression (Treg)

Antigen: APC T cell interaction with costimulation
Result – T cell activation, clonal expansion effector functions
T cell activation and tumors

Unlikely to occur
- loss of MHC I
- lack of co-stimulatory molecules
Bypass activation requirements and TCR

**“CAR” Chimeric Antigen Receptor**

- **Bypass activation requirements and TCR**

- **tumor antigen binding domain**

- **“CAR” Chimeric Antigen Receptor**

- **CD8a Leader:**
- **CD8a TM:**
- **CD28 TM:**

- **Signal domains:**
  - **1 kb**
  - **2 kb**

- **Zeta:**
- **BBz:**
- **28z:**
- **28BBz:**

- **SS1 scFv**
- **CD8a hinge**
- **4-1BB**
- **CD28**
- **CD3ζ**

- **sfv-ζ**

- **CD28**
- **4-1BB**
- **CD3**

- **Graph:**
  - **tumor volume (mm^3)**
  - **days post tumor injection**
  - **saline**
  - **CD19-28BBz IT**
  - **SS1-28BBz IP**
  - **SS1-28BBz IV**
  - **SS1-28BBz IT**
T cell activation and tumors

Unlikely to occur
- loss of efficient tumor cell killing
- lack of co-stimulatory molecules

TCR

Tumor antigen

tumor cell

co-stimulation required for full activation

CD8+ T cell
## CAR activity

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Other methods of engineering T cells
Adoptive Immunotherapy - Summary
Not limited to CD8 T cells
Response rates (not cure rate)

1994 – 34% with TIL

49% with TIL + immunodepletion

72% with TIL + immunodepletion + TBI
Why does adoptive immunotherapy fail?

- poor antigen display by tumor
- antigen loss
- hostile tumor microenvironment
- immune tolerance
- regulatory T cells
- insufficient numbers/persistence of cells
- inability to access tumors – limited lymphocyte trafficking
Antigen Loss
Cell Transfer Therapy for Cancer: Lessons from Sequential Treatments of a Patient With Metastatic Melanoma


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‡Diagnostic Radiology Department, Clinical Center, National Cancer Institute, National Institute of Health, Bethesda, Maryland
A unique case study

- 1997 – 27 year old female with melanoma of the eyelid
- excised, but recurred in 1999
- treated with superficial parotidectomy, cervical lymph node dissection, 60 Gy to her face and neck, and biochemotherapy – chemo + IL-2 + alpha-interferon
- disease progressed referred to NCI in 2000
Treated with peptide vaccine and IL-2

gp 100 antigen

November 2000
Adoptive transfer x5

Treatment #1 – autologous lymphocytes
- 1 source – PBL
- reactive gp100
- immunodepletion prior
- $1 \times 10^{10}$ cells injected i.v.
- minimal response

January 2001
Lymphodepletion improves response

Treatment #2, #3 – autologous lymphocytes
- 2 sources – PBL + TIL
- reactive gp100, MART-1
- immunodepletion prior to #3
- \( \sim 4 \times 10^{10} \) cells injected i.v.
- improved response

March 2001
Lymphocyte trafficking is important

Treatment #4 –

**autologous lymphocytes**  
- 2 sources – PBL + TIL  
- reactive gp100, MART-1  
- immunodepletion prior  
- ~4x10^{10} cells injected i.a.  
- much improved response

May 2001
Antigen loss is real and leads to tumor escape

Treatment #5 –
- autologous lymphocytes
  - 2 sources – PBL + TIL
  - reactive gp100, MART-1
  - immunodepletion prior
- 1 x 10^10 cells injected i.a.
- no response
- tumor biopsy revealed a single point mutation and loss of HLA-A2 antigens

- Patient refused surgery for growing nodules of neck
- died of progressive melanoma December 2001

- no response
Hostile Tumor Microenvironment

- Adenosine
- Indoleamine 2,3 dioxygenase (IDO)
- hypoxic
- acidotic
- lack of co-stimulation/cytokines
Adenosine

- damaged tissues – nucleotidases
- ATP/AMP converted to adenosine
- A1, A2A, A2B, and A3 receptors
- inhibits activation and expansion of T cells
- “Hellstrom paradox”
- normally a protective mechanism

8PNAS 2006; 103(35): 13132-13137.
Adenosine

\[ \text{Adenosine} \]

\[ A_1 R \quad \downarrow \quad A_2a R \quad \uparrow \quad A_2b R \quad \downarrow \quad A_3 R \]

\[ G_1/G_0 \quad \downarrow \quad G_2/G_{olf} \quad \downarrow \quad G_4/G_q \quad \downarrow \quad G_i/G_q \]

\[ \downarrow \text{cAMP} \quad \uparrow \text{cAMP} \quad \uparrow \text{p38 MAPK} \quad \uparrow \text{ERK 1/2} \]

\[ \uparrow \text{PLC/} \uparrow \text{Ca}^{2+} \quad \downarrow \text{PLC/} \uparrow \text{Ca}^{2+} \]

\[ \text{Intracellular} \quad \text{Extracellular} \]

\[^8\text{Int J Oncol 2008; 32(3): 527-35.}\]
Adenosine

- inhibits both activation and expansion
- SHP-2 – tyrosine phosphatase
- inhibits P56 and ZAP-70

Adenosine

- Tregs – CD39 and CD73 – sequentially catalyze to generate adenosine
Adenosine

Effects of adenosine receptor-mediated, so . . . .
Adenosine

Adenosine Inhibits Anti-Tumor Effects of T cells

Genetic targeting

Antagonism

"Rescue" of Anti-Tumor Effector Functions of T cells

- physiologic doses

8PNAS 2006; 103(35): 13132-13137.
Indolemine 2,3 dioxygenase (IDO)
Indolemine 2,3 dioxygenase (IDO)

- metabolizes tryptophan
- responsible for tumor tolerance
  - primary tumor site
  - draining lymph node
- normally – upregulated in APC in response to INF-γ (negative feedback loop)
- major mechanism of Treg

Tryptophan catabolic pathways

- Tryptophan
  - TDO or IDO
  - N-formylkynurenine
    - Kynurenic acid
    - 3-hydroxy-kynurenine
      - 3-hydroxy-anthranilic acid
        - Quinolinic acid
        - Picolinic acid
          - NAD+
  - TPH
  - 5-hydroxy-tryptophan
    - Serotonin
Indolemin 2,3 dioxygenase (IDO)

- IDO dysregulated in tumor due to Bin1 loss
- 1MT inhibitor of IDO in clinical trials

Indolemine 2,3 dioxygenase (IDO)

Mechanism of IDO gene regulation

Indolemine 2,3 dioxygenase (IDO)

\[ \text{Trp} \downarrow \rightarrow \text{Kyn and trp metabolites} \]

\[ \text{T}_{\text{reg}} \uparrow \rightarrow \text{Effector T cell proliferation} \]

\[ \text{Effector T cell apoptosis} \]

\[ \downarrow \]

Impairment of anti-tumor immune responses

\(^9\text{Immun Rev 2008; 222: 206-221.}\)
Feed forward mechanism – Treg ↔ DC

- typically CD4(+)CD25(+)FOXP3(+)
- can be antigen specific
- mediated via IDO to affect other T cells and APC
- depletion prior to AT has proven efficacy
Senescence
Insufficient numbers/persistence of transferred cells

- T cells close to senescence by time of adoptive transfer

Insufficient numbers/persistence of transferred cells

- less differentiated “younger” better?

Insufficient numbers/persistence of transferred cells

- Persistence in vivo beneficial
- Telomere regulation via “shelterin”
- control telomerase activity → “immortal” T cell?

\(^{11}\text{J Immunother. 2007; 30(1): 123-129.}\)
Limited trafficking into tumor
Limited lymphocyte trafficking to tumor

- cell mediated killing requires direct contact
- tumor vessels are grossly abnormal
- normal physiologic mechanisms of lymphocyte trafficking nearly impossible
Abnormal tumor vasculature as means of immune escape

- **Healthy vessel**
  - Well organized
  - Defined arterioles and venules
  - Regularly distributed
  - Non-dilated
  - Non-permeable
  - Mature and coated with mural cells
  - Low interstitial pressure
  - Complete basement membrane
  - Endothelial cell and mural cell
  - Appropriate expression of markers
  - Normal rate of blood flow

- **Tumor vessel**
  - Disorganized
  - Undefined arterioles and venules
  - Unevenly distributed
  - Dilated
  - Highly permeable
  - Premature and lack of mural cells
  - High interstitial pressure
  - Lack basement membrane
  - Mosaic cells
  - High or low expression of markers
  - Sluggish blood flow
Lymphocyte trafficking in preclinical models

Current models of trafficking of lymphocytes
selectins $\rightarrow$ chemokine receptors $\rightarrow$ integrins

Methods of following lymphocyte movement -

- radiolabeled cells
- cell surface marker variation
- fluorescent tracking dyes
- genetic variations
How does one study T-cell trafficking in vivo?

Measure cell accumulation and the impact of receptor blockade.

Initial entry from PB and intravascular pools.
Late entry from cells proliferating in 2º lymphoid organs.

RECRUITMENT

Must separate initial from late recruitment since the cell types, the pertinent receptors and the impact of the tumor microenvironment may change.

Late entry from cells proliferating in 2º lymphoid organs.

TURNOVER

Must separate recruitment from turnover so that one can determine whether an experimental intervention impacts one or both processes.

In situ apoptosis.

Efflux.

In situ proliferation.

Must separate recruitment from turnover so that one can determine whether an experimental intervention impacts one or both processes.

How does one study T-cell trafficking in vivo?
Inject calcein-labeled cells
Intravital microscopy (IVM)
Intravital microscopy (IVM)
Intravital microscopy (IVM) lymph node
Intravital microscopy (IVM) tumor
A picture is worth a thousand graphs . . .

wild type  PE-KO mice
Why does adoptive immunotherapy fail?

- poor antigen display by tumor
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- hostile tumor microenvironment
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